Zusammenfassung

An zeitgleich erhobenem Material aus verschiedenen Habitaten zweier Gebiete im ostelbischen Tiefland werden die Spezifika einer mit *Sorex araneus* L. syntop lebenden verwandten „Schmalform“ (*Sorex* spec.) in Farbe, Form, Meßwerten, Bionomie und Ökologie aufgezeigt und bewertet (Tab. 1–6). Ein Vergleich mit weiteren, regional nicht nachgewiesenen Arten *(S. coronatus, S. caecutiens, S. isodon)* belegt nahe Verwandtschaft (Färbung, Körpergröße, Reproduktionszyklus, Habitatwahl) zu *Sorex isodon* Turov, 1924. Von *Sorex isodon ruthenus* Stroganow, 1936 hebt sich die „Märkische Schmalspitzmaus“ *Sorex isodon marchicus* ssp. nova durch kürzeren Schwanz und Fuß sowie geringere Schädelmaße ab (Tab. 7–8).

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Cytogenetic studies on the mitotic and meiotic chromosomes of *Micromys minutus* (Rodentia, Murinae)

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Abstract

Cytogenetical investigations demonstrate a diploid chromosome number of 2n = 68. All chromosomes are acrocentric except the autosome pair no. 1 and the very small Y chromosome which both are sub- to metacentric.

Approximately 32 % of the karyotype consists of constitutive heterochromatin which is localized in the pericentromeric regions of all acrocentric chromosomes and exhibits a very bright fluorescence

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after counterstaining with distamycin A/DAPI. Specific stainings of the constitutive heterochromatin in the chromosomes of male meiotic cells reveal an exceptional pairing configuration of the X- and the Y chromosomes. Only a small euchromatic region near the centromere of the otherwise entirely heterochromatic Y chromosome pairs with a segment located near the centromere of the long arm of the acrocentric X chromosome. This leads to a trefoil-like structure.

Introduction

The harvest mouse *Micromys minutus* (Rodentia, Murinae) is smaller than most other murine species (average length: 6 cm). This unique species has a climbing way of life and builds spherical nests in bushes a short distance above ground for breeding their offsprings. Its habitat extends from eastern Kantabria and South England throughout Europe and Asia up to the Japanese islands, Assam and Burma (Böhme 1978). The genus *Micromys* is distinguished from the other members of the rodent subfamily Murinae not only by its behavorial biology, morphology, anatomy and cytology but also by its diploid chromosome number of 2n = 68, found in the subspecies *M. minutus soricinus* (Middle Europe), *M. minutus batavori* (Western Sibiria) and *M. minutus takasogenesis* (Japan) (Kral 1971). Only 4 % of the Murinae species possess more than 60 chromosomes (average chromosome number: 2n = 42) (Matthey 1973).

In the present study, the mitotic and meiotic chromosomes of *M. minutus* were examined with various banding techniques and some cytogenetic peculiarities are presented.

Material and methods

5 males and 5 females of *Micromys minutus,* caught in South Germany in the vicinity of Munich, were examined. 1 hour prior to sacrifice with diethyl-ether the animals were intraperitoneally injected with 0.4 ml of an 0.03 % Colcemid solution. Biopsies from lung and peritoneum were obtained and fibroblast cultures initiated in MEM enriched with 16 % fetal calf serum. After 3–4 weeks chromosome preparations were performed according to standard methods (Wolf 1974). Mitotic chromosomes were also obtained from bone marrow preparations according to the method of Evans et al. (1964).

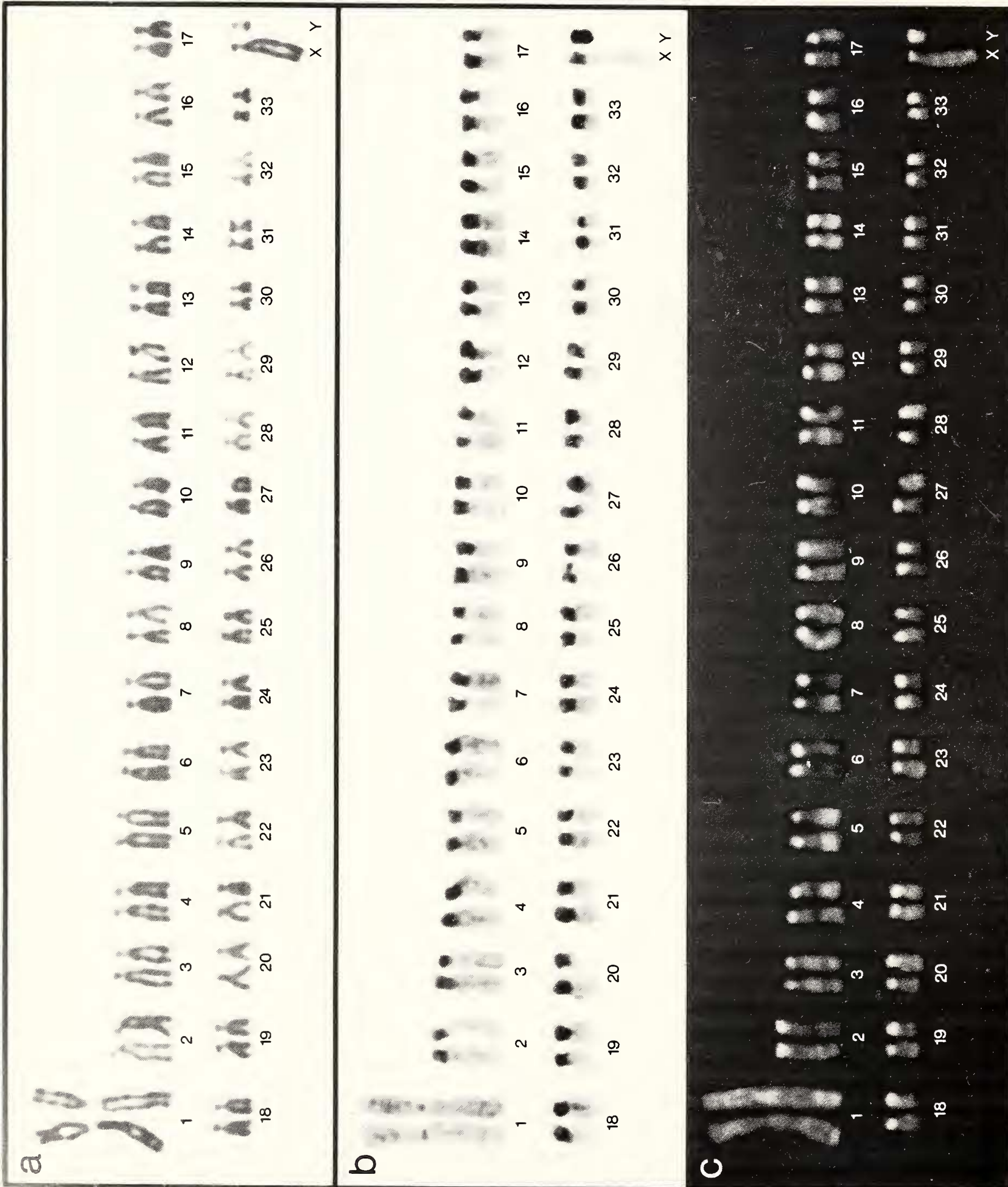
The preparations were stained with Giemsa (5–10 min in a 4 % Giemsa solution) or counterstained with distamycin A/DAPI (Schweizer et al. 1978) after pre-identifying the chromosomes by Q-banding according to the method of Caspersson et al. (1970). C-banding was performed according to the technique of Sumner (1972). Pre-identification of the chromosomes with quinacrine mustard was necessary because the acrocentric autosomes could not be identified with certainty in the Giemsa-stained, distamycin A/DAPI-counterstained and C-banded slides.

Length measurements of the chromosomes and their corresponding C-bands were made on photographed metaphases.

Results

The Giemsa-stained karyotype of a male animal is shown in Fig. 1a. The karyotype of *Micromys minutus* consists of 68 chromosomes. All chromosomes are acrocentric except chromosome pair 1 and the nearly metacentric Y chromosome. The submetacentric autosome pair 1 distinguishes from the other chromosomes of the complement; it is nearly twice as long as the others. Except the X chromosome which is the longest acrocentric chromosome in the karyotype none of the acrocentric chromosomes can be identified with certainty. This fact makes it necessary to pre-identify the chromosomes by banding with quinacrine mustard. The length of pair 2 to 33 decreases only slightly. In two of the males a remarkable polymorphism was found in the short arms of chromosome pair 27. In one of the homologues the short arm was so reduced that the chromosome exhibited a telomeric morphology (Fig. 1). The Y chromosome is the smallest chromosome in the karyotype and its long arm stains distinctly darker than the short arm and the rest of the karyotype.

In Fig. 1b the C-banded chromosomes of *M. minutus* pre-identified with quinacrine mustard are presented. Large blocks of constitutive heterochromatin in the centromeric regions and in the short arms of all the acrocentric chromosomes are obvious. The smallest amount of constitutive heterochromatin can be found in chromosome pair 1. The Y chromosome seems to be totally heterochromatic. However, in some high quality metaphases a tiny euchromatic region localized in the proximity of the centromere was observed.



The banding pattern of the chromosomes after counterstaining with distamycin A/DAPI is demonstrated in Fig. 1c. The constitutive heterochromatin in the short arms of all the acrocentric chromosomes exhibits a bright fluorescence. In the Y chromosome only the heterochromatin in the long arm fluoresces brightly. In the autosome pair no. 1 the centromeric constitutive heterochromatin exhibits no distamycin A/DAPI positive fluorescence.

Fig. 2 demonstrates diakineses of male meiosis, after C-banding (2a) and after counterstaining with distamycin A/DAPI (2c). The autosomal bivalents have a rod to ring-like structure, whereas in all investigated diakineses the XY-bivalent of *M. minutus* exhibits a peculiar trefoil-like structure. This unique structure is the result of the pairing of the pericentromeric region of the Y chromosome with the pericentromeric region of the X chromosome. The interpretation of the exact arrangement of the sex-bivalent was possible by C-banding and distamycin A/DAPI staining (2b, 2d). Two of the three heterochromatic blocks in the XY-bivalent are contributed by the two arms of the metacentric Y chromosome, the third one by the short arm of the X chromosome. One of these heterochromatic regions always orientates in the opposite direction of the euchromatic long arm of the X chromosome. The distamycin A/DAPI-counterstaining reveals that the brightly fluorescing arm of the Y chromosome is always found in close vicinity to the brightly fluorescing arm of the X chromosome, whereas the distamycin A/DAPI negative arm of the Y chromosome extrudes out of the formation of the XY-bivalent. These configurations were found in more than 300 diakineses examined.

The length measurements performed on photographed C-banded metaphases revealed that 31 % of the total karyotype consists of constitutive heterochromatin.

Discussion

The diploid chromosome number of 2n = 68 found by KRAL (1971) for *M. minutus* was confirmed in the present study (Fig. 1). The high chromosome number may indicate a special position of *M. minutus* within the subfamily Murinae (average chromosome number: 2n = 42; MATTHEY 1973). To our knowledge only in 4 African Murinae such high diploid chromosome numbers were found: *Arvicanthis abyssinicus* (2n = 64), *Acomys subspinosus* (2n = 64), *Acomys russatus* (2n = 66) and *Lophuromys acquilus laticeps* (2n = 70) (MATTHEY 1973). The bright distamycin A/DAPI fluorescence of the constitutive heterochromatin indicates that the repetitive DNA localized in these heterochromatic regions is enriched in AT base pairs. There are only very few mammalian karyotypes that exhibit such a high amount of distamycin A/DAPI heterochromatin as *M. minutus* (SCHWEIZER 1983). This bright fluorescence that can be seen also in interphase nuclei predestinates this species for investigations on the arrangement of the centromeres in the interphase stage.

Another peculiarity of the *M. minutus* karyotype is the high amount of heterochromatin in the karyotype (31.8 %), found by length measurements on photographed metaphases. Thus the analysis of eight mammalian species belonging to eight different genera (HSU and ARRIGHI 1971) revealed that approximately 20 % of the genome in all eight species consists of constitutive heterochromatin. This high content of heterochromatin in the genome of *M. minutus* resembles the situation in a few species of the North American

Fig. 1. Karyotypes of a male animal of *Micromys minutus* with the diploid chromosome number of 2n = 68. In a Giemsa-stained chromosomes are presented. Note the heteromorphism in autosome pair no 27. The C-banded karyotype (b) shows large blocks of constitutive heterochromatin in the pericentromeric regions and the short arms of all the acrocentric chromosomes. The Y chromosome seems to be totally heterochromatic, whereas the autosome pair no 1 exhibits only a small C-banded segment

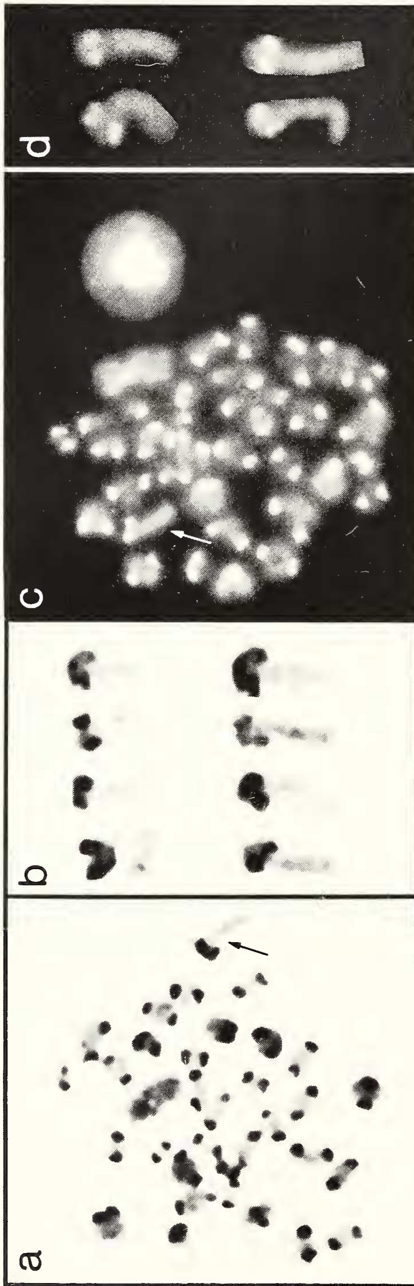


Fig. 2. Diakinetic stages (a, c) and selected sex-bivalents (b, d) of a male *Micromys minutus* stained with the C-banding technique (a, b) and by distamycin A/DAPI-counterstaining (c, d). The arrows in a and c point to the XY-bivalents. Note the trefoil-like pairing arrangement of the XY-bivalent (b, d). It can be clearly seen that the brightly fluorescing short arm of the Y chromosome pairs in close vicinity to the brightly fluorescing arm of the X chromosome; the distamycin A/DAPI negative arm of the Y chromosome is localized opposite to the euchromatic long arm of the X-chromosome. Note that in the spermatid nucleus all the brightly fluorescing heterochromatic regions are fused to a single chromocenter

genus *Peromyscus* (Rodentia, Cricetinae), where e.g. in *P. eremicus* the DNA amount is increased by 36 % compared to most mammalian species. Large amounts of heterochromatin in the short arms of all chromosomes of this species are responsible for the excess of DNA, whereas the euchromatic long arms are unaltered (Pathak et al. 1973; Deaven et al. 1977).

The most striking fact in the present cytogenetic studies on *M. minutus* is the peculiar pairing of the XY-bivalent. In all investigated mammalian species belonging to all main orders the X and Y always show end-to-end-pairing and never a lateral arrangement (White 1973). The current view on meiotic behaviour of mammalian sex chromosomes is that the end-to-end arrangement is the result of a partial synapsis followed by a single crossover with subsequent terminalization (for review see Solari 1974; Burgoyne 1982). A genetically homologous region existing in both chromosomes is supposed to mediate the pairing. The cytogenetic analysis of the XY-bivalent of *M. minutus* leads to the conclusion that in this species the genetic homologous segments in the X- and the Y chromosome are not localized terminally. On the contrary, the trefoil-like structure of the XY-bivalent reveals that the tiny euchromatic region close to the centromere in the Y chromosome pairs with an euchromatic segment adjacent to the heterochromatin in the long arm of the X chromosome. In this pairing process the constant orientation of the two sex chromosomes within the bivalent is determined by their homologous DNA sequences.

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Zusammenfassung

Untersuchungen der mitotischen und meiotischen Chromosomen von Micromys minutus (Rodentia, Murinae)

Zytogenetische Untersuchungen der Zwergmaus *Micromys minutus* (Rodentia, Murinae) ergaben eine diploide Chromosomenzahl von 2n = 68. Mit Ausnahme des submetazentrischen Autosomenpaars No. 1 und des sehr kleinen metazentrischen Y-Chromosoms sind alle anderen Chromosomen akrozentrisch. Das gesamte konstitutive Heterochromatin, welches hauptsächlich in den perizentromerischen Regionen und in den kurzen Armen der Chromosomen lokalisiert ist, zeichnet sich durch eine sehr helle Fluoreszenz nach Distamycin A/DAPI Doppelfärbung aus. Derart hell fluoreszierendes Heterochromatin, das in diesem Ausmaße nur bei sehr wenigen Säugern gefunden wird, kann als Marker für die Lage der Zentromere in den Interphasenkernen herangezogen werden. Längenmessungen an photographierten, C-gebänderten Chromosomen ergaben einen außergewöhnlich hohen Anteil an konstitutivem Heterochromatin.

Die spezifische Färbung des konstitutiven Heterochromatins in den Chromosomen der männlichen Meiose zeigt eine außergewöhnliche Paarungskonfiguration zwischen X- und Y-Chromosom. Eine sehr kleine zentromernahe, euchromatische Region im sonst völlig heterochromatischen Y-Chromosom paart mit einem zentromernahen Segment im langen Arm des akrozentrischen X-Chromosoms. Hierdurch entsteht eine Kleeblatt-ähnliche Anordnung. Diese Paarungsanordnung steht im Gegensatz zu der Meiose von anderen Säugetieren, in der die X- und Y-Chromosomen eine terminale Paarung miteinander eingehen.

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